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Evaluation of collagen release from cultured human fibroblasts via ultrasonic microscope

超音波顕微鏡を用いたヒト真皮線維芽細胞からのコラーゲン放出

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1. Background

Human dermal fibroblasts in the dermis produce extracellular matrices such as collagen, which provide elasticity to the skin. *l*-Ascorbic acid is a fibrillogenesis factor for collagen, but it is easily oxidized, and it is important to develop effective and stable derivatives. In this study, cultured human dermal fibroblasts treated with magnesium phosphate-L-ascorbate (MAP) or dehydroascorbic acid (DHA), both of which are oxidation-resistant vitamin C derivatives, were observed using ultrasound microscopy, which allows for non-invasive, non-staining observation, to compare the effects on cell proliferation and collagen fibril production. We compared and examined the effects on cell proliferation and collagen fibril production. In addition, we quantified the amount of collagen using the ELISA method and attempted to make further comparisons with the data obtained from the cells by performing ultrasound measurements of collagen alone.

2. Material and Methods

Human neonatal-derived dermal fibroblasts were proliferated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 10 ml/L Penicillin-Streptomycin with 10^4 cells/dish primarily in the CO_2 incubator, 37℃. For acoustic or optical observation, we used the PS-film dish (Honda Electronics Co., Ltd.) At 2 days in vitro (DIV), cultured fibroblast was applied with MAP, or DHA, and observed to 8 DIV.

We observed the change of fibroblast cells using ultrasound microscopy with a center frequency of 320 MHz at 8 DIV, and phase-contrast microscopy daily. After acoustic observation, samples fixed with 4% paraformaldehyde were stained with anti-actin and anti-type I collagen antibodies and observed with a confocal microscope. ELISA for Procollagen end peptide (PIP) was used to quantify released collagen in the culture medium.

3. Results and Discussion

Ultrasound imaging revealed a medial acoustic impedance region around the cells at 8 DIV. That intensity was higher than the culture medium and lower than intracellular cytoskeletons (Fig. 1). The MAP administration increased the area of this medial acoustic impedance region, especially 0.2 mM MAP administration showed a significant effect, suggesting that it is one of the criteria for optimal concentration. DHA administration showed no significant effect to control. Quantification of collagen amount by ELISA showed the same trend as in ultrasound microscopy (Fig.2). In cultured human dermal fibroblasts up to DIV8, cell proliferative capacity showed no significant change among MAP treatment, DHA treatment, and control using the BCA protein assay method.

The impedance of cross-linking collagen molecules, which showed higher than it of uncross-linking collagen molecules, was likely the extracellular acoustic impedance around the cells.

It suggests that acoustic imaging is an effective means of visualizing released collagen fibrillogenesis in living cells because this method is non-invasive, non-staining, and can be observed rapidly.

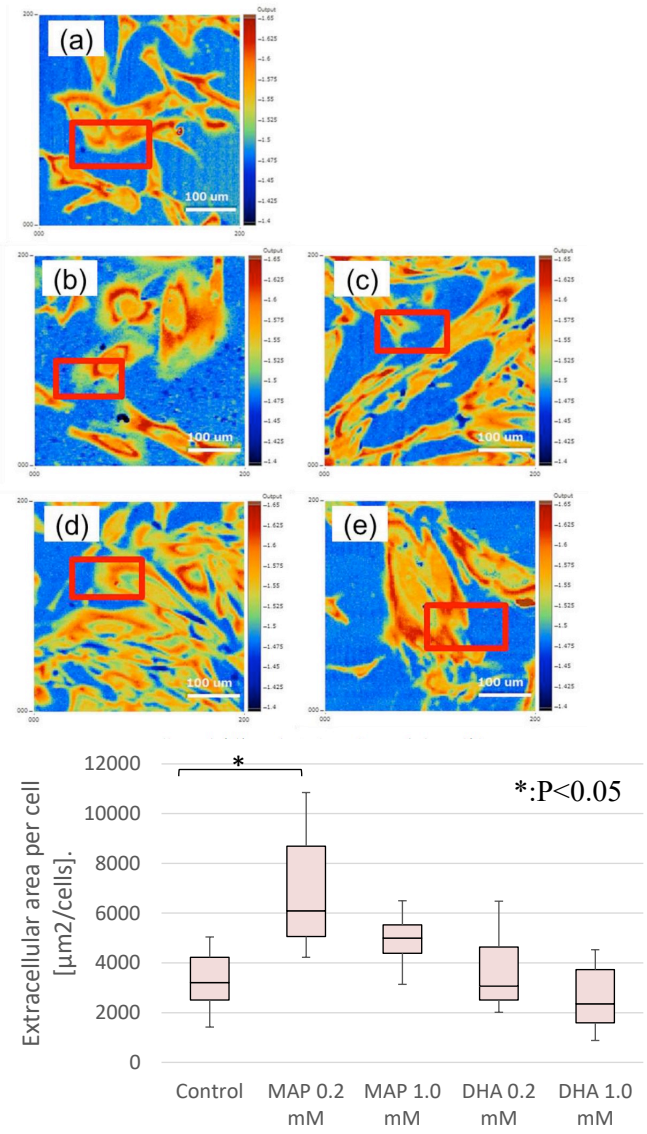

Fig.1 Ultrasound microscopy images of cultured human skin fibroblasts (DIV8)
(a) Control (b) MAP 0.2 mM dose (c) MAP 1 mM dose (d) DHA 0.2 mM dose (e) DHA 1.0 mM dose(Scale Bar=100 µm)
(f) Extracellular bass per cell area of impedance region (Dunnet test *;P＜0.05)

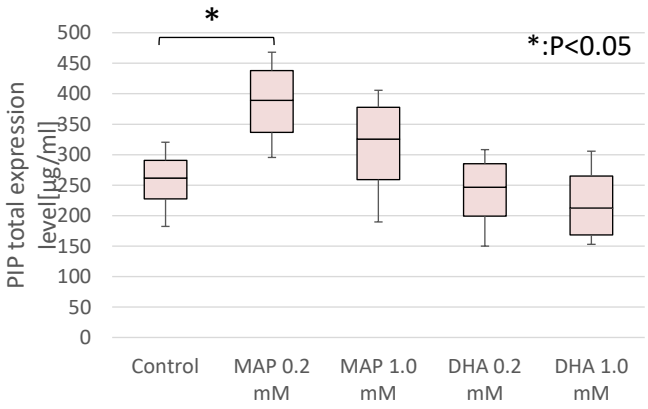

Fig.2 Total expression level of PIP in the culture medium using ELISA

4. Conclusion

The impedance of the extracellular region was identified as the cross-linked collagen using ultrasound microscopy in cultured human dermal fibroblasts. This area was increased the most with MAP 0.2 mM administration, which was similar to the result of collagen quantification by ELISA. We suggest ultrasound imaging would be useful for visualizing the extracellular collagen release from living cells.

References

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